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(71) Applicant *(for all designated States except US)*: THE JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE [US/US]; 720 Rutland Avenue, Baltimore, MD 21205 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: LEE, Se-Jin [US/US]; 6711 Chokeberry Road, Baltimore, MD 21209 (US). ESQUELA, Aurora, F. [US/US]; Apartment 1B, 853 West University Parkway, Baltimore, MD 21210 (US).

(74) Agents: HAILE, Lisa, A. et al.; Fish & Richardson P.C., Suite 1400, 4225 Executive Square, La Jolla, CA 92037 (US).

(54) Title: GROWTH DIFFERENTIATION FACTOR-12

(57) Abstract

Growth differentiation factor-12 (GDF-12) is disclosed along with its polynucleotide sequence and amino acid sequence. Also disclosed are diagnostic and therapeutic methods of using the GDF-12 polypeptide and polynucleotide sequences.

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GROWTH DIFFERENTIATION FACTOR-12

This is a continuation-in-part application of U.S. Serial No. 08/274,215, filed on July 13, 1994.

BACKGROUND OF THE INVENTION

1. *Field of the Invention*

The invention relates generally to growth factors and specifically to a new member of the transforming growth factor beta (TGF-β) superfamily, which is denoted, growth differentiation factor-12 (GDF-12).

2. *Description of Related Art*

The transforming growth factor β (TGF-β) superfamily encompasses a group of structurally-related proteins which affect a wide range of differentiation processes during embryonic development. The family includes, Mullerian inhibiting substance (MIS), which is required for normal male sex development (Behringer, *et al.*, *Nature*, 345:167, 1990), *Drosophila* decapentaplegic (DPP) gene product, which is required for dorsal-ventral axis formation and morphogenesis of the imaginal disks (Padgett, *et al.*, *Nature*, 325:81-84, 1987), the *Xenopus* Vg-1 gene product, which localizes to the vegetal pole of eggs ((Weeks, *et al.*, *Cell*, 51:861-867, 1987), the activins (Mason, *et al.*, *Biochem, Biophys. Res. Commun.*, 135:957-964, 1986), which can induce the formation of mesoderm and anterior structures in *Xenopus* embryos (Thomsen, *et al.*, *Cell*, 63:485, 1990), and the bone morphogenetic proteins (BMPs, osteogenin, OP-1) which can induce *de novo* cartilage and bone formation (Sampath, *et al.*, *J. Biol. Chem.*, 265:13198, 1990). The TGF-βs can influence a variety of

differentiation processes, including adipogenesis, myogenesis, chondrogenesis, hematopoiesis, and epithelial cell differentiation (for review, see Massague, *Cell* 49:437, 1987).

The proteins of the TGF-β family are initially synthesized as a large precursor protein which subsequently undergoes proteolytic cleavage at a cluster of basic residues approximately 110-140 amino acids from the C-terminus. The C-terminal regions, or mature regions, of the proteins are all structurally related and the different family members can be classified into distinct subgroups based on the extent of their homology. Although the homologies within particular subgroups range from 70% to 90% amino acid sequence identity, the homologies between subgroups are significantly lower, generally ranging from only 20% to 50%. In each case, the active species appears to be a disulfide-linked dimer of C-terminal fragments. Studies have shown that when the pro-region of a member of the TGF-β family is coexpressed with a mature region of another member of the TGF-β family, intracellular dimerization and secretion of biologically active homodimers occur (Gray, A., and Maston, A., *Science*, 247:1328, 1990). Additional studies by Hammonds, *et al.*, (*Molec. Endocrin.* 5:149, 1991) showed that the use of the BMP-2 pro-region combined with the BMP-4 mature region led to dramatically improved expression of mature BMP-4. For most of the family members that have been studied, the homodimeric species has been found to be biologically active, but for other family members, like the inhibins (Ling, *et al., Nature*, 321:779, 1986) and the TGF-βs (Cheifetz, *et al., Cell*, 48:409, 1987), heterodimers have also been detected, and these appear to have different biological properties than the respective homodimers.

Identification of new factors that are tissue-specific in their expression pattern will provide a greater understanding of that tissue's development and function.

SUMMARY OF THE INVENTION

The present invention provides a cell growth and differentiation factor, GDF-12, a polynucleotide sequence which encodes the factor, and antibodies which are immunoreactive with the factor. This factor appears to relate to various cell proliferative disorders, especially those involving liver cells.

Thus, in one embodiment, the invention provides a method for detecting a cell proliferative disorder of liver origin and which is associated with GDF-12. In another embodiment, the invention provides a method for treating a cell proliferative disorder by suppressing or enhancing GDF-12 activity.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 shows a Northern blot of RNA prepared from adult tissues probed with a murine GDF-12 probe.

FIGURE 2 shows the partial nucleotide and predicted amino acid sequence of human GDF-12.

FIGURE 3 shows the full length nucleotide and predicted amino acid sequence of human GDF-12.

FIGURE 4 shows amino acid sequence homologies between human GDF-12 and different members of the TGF-β superfamily. Numbers represent amino acid sequence identities between GDF-12 and the indicated family member calculated from the first conserved cysteine to the C-terminus.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a growth and differentiation factor, GDF-12 and a polynucleotide sequence encoding GDF-12. GDF-12 is expressed specifically in liver. In one embodiment, the invention provides a method for detection of a cell proliferative disorder of liver origin which is associated with GDF-12 expression. In another embodiment, the invention provides a method for treating a cell proliferative disorder by using an agent which suppresses or enhances GDF-12 activity.

The TGF-β superfamily consists of multifunctional polypeptides that control proliferation, differentiation, and other functions in many cell types. Many of the peptides have regulatory, both positive and negative, effects on other peptide growth factors. The structural homology between the GDF-12 protein of this invention and the members of the TGF-β family, indicates that GDF-12 is a new member of the family of growth and differentiation factors. Based on the known activities of many of the other members, it can be expected that GDF-12 will also possess biological activities that will make it useful as a diagnostic and therapeutic reagent.

In particular, the expression pattern of GDF-12 suggests that GDF-12 possesses activities that will make it useful for the treatment of various diseases involving the liver. For example, when GDF-12 functions to stimulate the growth or differentiation of liver cells, GDF-12 could be used for the treatment of disease states in which the function of the liver is compromised, such as in hepatitis or cirrhosis. Although liver tissue has the capacity to regenerate, GDF-12 could potentially accelerate the normal regenerative process or promote the process in disease states in which the regenerative process is suppressed. Similarly, GDF-12 could be useful for maintaining liver cells or tissue in culture prior to transplantation or for stimulating the growth of liver cells following transplantation; in this regard, because liver cells may be used as a vehicle for delivering genes to liver for gene therapy, GDF-12

could be useful for maintaining or expanding liver cells in culture during or after the introduction of particular genes or for stimulating the growth of these cells following transplantation.

Alternatively, when GDF-12 functions as a growth inhibitor, GDF-12 could be used to create cell proliferative disorders involving liver cells, such as hepatocellular carcinoma. Indeed, one member of this superfamily, namely, inhibin alpha, has been shown to function as a tumor suppressor gene, and another member of this superfamily, namely, Mullerian inhibiting substance, has been shown to be capable of inhibiting the growth of tumor cells both *in vitro* and *in vivo*.

This high specificity of GDF-12 expression also suggests potential applications of GDF-12 as a diagnostic tool. In particular, because GDF-12 encodes a secreted factor, levels of GDF-12 could be used to monitor liver function or to detect the presence of neoplasms involving liver cells. In this regard, another member of this family, namely, inhibin, has been shown to be useful as a marker for ovarian granulosa cell tumors.

The term "substantially pure" as used herein refers to GDF-12 which is substantially free of other proteins, lipids, carbohydrates or other materials with which it is naturally associated. One skilled in the art can purify GDF-12 using standard techniques for protein purification. The substantially pure polypeptide will yield a single major band on a non-reducing polyacrylamide gel. The purity of the GDF-12 polypeptide can also be determined by amino-terminal amino acid sequence analysis. GDF-12 polypeptide includes functional fragments of the polypeptide, as long as the activity of GDF-12 remains. Smaller peptides containing the biological activity of GDF-12 are included in the invention.

The invention provides polynucleotides encoding the GDF-12 protein. These polynucleotides include DNA, cDNA and RNA sequences which encode GDF-

12. It is understood that all polynucleotides encoding all or a portion of GDF-12 are also included herein, as long as they encode a polypeptide with GDF-12 activity. Such polynucleotides include naturally occurring, synthetic, and intentionally manipulated polynucleotides. For example, GDF-12 polynucleotide may be subjected to site-directed mutagenesis. The polynucleotide sequence for GDF-12 also includes antisense sequences. The polynucleotides of the invention include sequences that are degenerate as a result of the genetic code. There are 20 natural amino acids, most of which are specified by more than one codon. Therefore, all degenerate nucleotide sequences are included in the invention as long as the amino acid sequence of GDF-12 polypeptide encoded by the nucleotide sequence is functionally unchanged.

Specifically disclosed herein is a partial cDNA sequence containing the active portion of the human GDF-12 coding sequence. One of skill in the art could now use this partial sequence to isolate the full length clones. The cDNA clone from which this sequence was obtained is likely to contain the entire coding sequence for GDF-12. The disclosed sequence corresponds to the C-terminal region of the GDF-12 polypeptide. The sequence begins with a putative proteolytic cleavage site, RARRR. Cleavage of the polypeptide at this site would generate an active C-terminal fragment 114 amino acids in length with a predicted molecular weight of 12,500.

The C-terminal region of GDF-12 following the putative proteolytic processing site shows significant homology to the known members of the TGF-β superfamily. The GDF-12 sequence contains most of the residues that are highly conserved in other family members (see FIGURE 1). Like the TGF-βs and inhibin βs, GDF-12 contains an extra pair of cysteine residues in addition to the 7 cysteines found in virtually all other family members. Among the known family members, GDF-12 is most homologous to Inhibin βB (50% sequence identity) (see FIGURE 4).

Minor modifications of the recombinant GDF-12 primary amino acid sequence may result in proteins which have substantially equivalent activity as compared to the GDF-12 polypeptide described herein. Such modifications may be deliberate, as by site-directed mutagenesis, or may be spontaneous. All of the polypeptides produced by these modifications are included herein as long as the biological activity of GDF-12 still exists. Further, deletion of one or more amino acids can also result in a modification of the structure of the resultant molecule without significantly altering its biological activity. This can lead to the development of a smaller active molecule which would have broader utility. For example, one can remove amino or carboxy terminal amino acids which are not required for GDF-12 biological activity.

The nucleotide sequence encoding the GDF-12 polypeptide of the invention includes the disclosed sequence and conservative variations thereof. The term "conservative variation" as used herein denotes the replacement of an amino acid residue by another, biologically similar residue. Examples of conservative variations include the substitution of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another, or the substitution of one polar residue for another, such as the substitution of arginine for lysine, glutamic for aspartic acid, or glutamine for asparagine, and the like. The term "conservative variation" also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid provided that antibodies raised to the substituted polypeptide also immunoreact with the unsubstituted polypeptide.

The polynucleotide encoding GDF-12 includes the nucleotide sequence in FIGURES 2 and 3 (SEQ ID NO:11 and NO:13, respectively), as well as nucleic acid sequences complementary to that sequence. A complementary sequence may include an antisense nucleotide. When the sequence is RNA, the deoxynucleotides A, G, C, and T of FIGURE 2 and 3 are replaced by ribonucleotides A, G, C, and U, respectively. Also included in the invention are fragments of the above-described nucleic acid sequences that are at least 15 bases in length, which is sufficient to permit the fragment to selectively hybridize to DNA that encodes the protein of FIGURES 2 and 3 (SEQ ID NO:12 and NO:14, respectively) under physiological conditions.

DNA sequences of the invention can be obtained by several methods. For example, the DNA can be isolated using hybridization techniques which are well known in the art. These include, but are not limited to: 1) hybridization of genomic or cDNA libraries with probes to detect homologous nucleotide sequences, 2) polymerase chain reaction (PCR) on genomic DNA or cDNA using primers capable of annealing to the DNA sequence of interest, and 3) antibody screening of expression libraries to detect cloned DNA fragments with shared structural features.

Preferably the GDF-12 polynucleotide of the invention is derived from a mammalian organism, and most preferably from a mouse, rat, or human. Screening procedures which rely on nucleic acid hybridization make it possible to isolate any gene sequence from any organism, provided the appropriate probe is available. Oligonucleotide probes, which correspond to a part of the sequence encoding the protein in question, can be synthesized chemically. This requires that short, oligopeptide stretches of amino acid sequence must be known. The DNA sequence encoding the protein can be deduced from the genetic code, however, the degeneracy of the code must be taken into account. It is possible to perform a mixed addition reaction when the sequence is degenerate. This includes a heterogeneous mixture of denatured double-stranded DNA. For such screening, hybridization is preferably

performed on either single-stranded DNA or denatured double-stranded DNA. Hybridization is particularly useful in the detection of cDNA clones derived from sources where an extremely low amount of mRNA sequences relating to the polypeptide of interest are present. In other words, by using stringent hybridization conditions directed to avoid non-specific binding, it is possible, for example, to allow the autoradiographic visualization of a specific cDNA clone by the hybridization of the target DNA to that single probe in the mixture which is its complete complement (Wallace, *et al., Nucl. Acid Res.*, 9:879, 1981).

Therefore, given a partial DNA sequence of the gene of interest, one of skill in the art would be able to prepare probes for isolation of a full length cDNA clone, without undue experimentation (see for example, Ausubel, *et al., Current Protocols in Molecular Biology*, Units 6.3-6.4, Greene Publ., 1994; Maniatis, *et al., Molecular Cloning,* Cold Spring Harbor Laboratories, 1982).

The development of specific DNA sequences encoding GDF-12 can also be obtained by: 1) isolation of double-stranded DNA sequences from the genomic DNA; 2) chemical manufacture of a DNA sequence to provide the necessary codons for the polypeptide of interest; and 3) *in vitro* synthesis of a double-stranded DNA sequence by reverse transcription of mRNA isolated from a eukaryotic donor cell. In the latter case, a double-stranded DNA complement of mRNA is eventually formed which is generally referred to as cDNA.

Of the three above-noted methods for developing specific DNA sequences for use in recombinant procedures, the isolation of genomic DNA isolates is the least common. This is especially true when it is desirable to obtain the microbial expression of mammalian polypeptides due to the presence of introns.

The synthesis of DNA sequences is frequently the method of choice when the entire sequence of amino acid residues of the desired polypeptide product is

known. When the entire sequence of amino acid residues of the desired polypeptide is not known, the direct synthesis of DNA sequences is not possible and the method of choice is the synthesis of cDNA sequences. Among the standard procedures for isolating cDNA sequences of interest is the formation of plasmid- or phage-carrying cDNA libraries which are derived from reverse transcription of mRNA which is abundant in donor cells that have a high level of genetic expression. When used in combination with polymerase chain reaction technology, even rare expression products can be cloned. In those cases where significant portions of the amino acid sequence of the polypeptide are known, the production of labeled single or double-stranded DNA or RNA probe sequences duplicating a sequence putatively present in the target cDNA may be employed in DNA/DNA hybridization procedures which are carried out on cloned copies of the cDNA which have been denatured into a single-stranded form (Jay, *et al., Nucl. Acid Res.*, 11:2325, 1983).

A cDNA expression library, such as lambda gt11, can be screened indirectly for GDF-12 peptides having at least one epitope, using antibodies specific for GDF-12. Such antibodies can be either polyclonally or monoclonally derived and used to detect expression product indicative of the presence of GDF-12 cDNA.

DNA sequences encoding GDF-12 can be expressed *in vitro* by DNA transfer into a suitable host cell. "Host cells" are cells in which a vector can be propagated and its DNA expressed. The term also includes any progeny of the subject host cell. It is understood that all progeny may not be identical to the parental cell since there may be mutations that occur during replication. However, such progeny are included when the term "host cell" is used. Methods of stable transfer, meaning that the foreign DNA is continuously maintained in the host, are known in the art.

In the present invention, the GDF-12 polynucleotide sequences may be inserted into a recombinant expression vector. The term "recombinant expression vector" refers to a plasmid, virus or other vehicle known in the art that has been manipulated by insertion or incorporation of the GDF-12 genetic sequences. Such expression vectors contain a promoter sequence which facilitates the efficient transcription of the inserted genetic sequence of the host. The expression vector typically contains an origin of replication, a promoter, as well as specific genes which allow phenotypic selection of the transformed cells. Vectors suitable for use in the present invention include, but are not limited to the T7-based expression vector for expression in bacteria (Rosenberg, *et al.*, *Gene*, 56:125, 1987), the pMSXND expression vector for expression in mammalian cells (Lee and Nathans, *J. Biol. Chem.*, 263:3521, 1988) and baculovirus-derived vectors for expression in insect cells. The DNA segment can be present in the vector operably linked to regulatory elements, for example, a promoter (e.g., T7, metallothionein I, or polyhedrin promoters).

Polynucleotide sequences encoding GDF-12 can be expressed in either prokaryotes or eukaryotes. Hosts can include microbial, yeast, insect and mammalian organisms. Methods of expressing DNA sequences having eukaryotic or viral sequences in prokaryotes are well known in the art. Biologically functional viral and plasmid DNA vectors capable of expression and replication in a host are known in the art. Such vectors are used to incorporate DNA sequences of the invention. Preferably, the mature C-terminal region of GDF-12 is expressed from a cDNA clone containing the entire coding sequence of GDF-12. Alternatively, the C-terminal portion of GDF-12 can be expressed as a fusion protein with the pro- region of another member of the TGF-β family or co-expressed with another pro- region (see for example, Hammonds, *et al.*, *Molec. Endocrin.* 5:149, 1991; Gray, A., and Mason, A., *Science*, 247:1328, 1990).

Transformation of a host cell with recombinant DNA may be carried out by conventional techniques as are well known to those skilled in the art. Where the host is prokaryotic, such as *E. coli*, competent cells which are capable of DNA uptake can be prepared from cells harvested after exponential growth phase and subsequently treated by the $CaCl_2$ method using procedures well known in the art. Alternatively, $MgCl_2$ or RbCl can be used. Transformation can also be performed after forming a protoplast of the host cell if desired.

When the host is a eukaryote, such methods of transfection of DNA as calcium phosphate co-precipitates, conventional mechanical procedures such as microinjection, electroporation, insertion of a plasmid encased in liposomes, or virus vectors may be used. Eukaryotic cells can also be cotransformed with DNA sequences encoding the GDF-12 of the invention, and a second foreign DNA molecule encoding a selectable phenotype, such as the herpes simplex thymidine kinase gene. Another method is to use a eukaryotic viral vector, such as simian virus 40 (SV40) or bovine papilloma virus, to transiently infect or transform eukaryotic cells and express the protein. (see for example, *Eukaryotic Viral Vectors*, Cold Spring Harbor Laboratory, Gluzman ed., 1982).

Isolation and purification of microbial expressed polypeptide, or fragments thereof, provided by the invention, may be carried out by conventional means including preparative chromatography and immunological separations involving monoclonal or polyclonal antibodies.

The GDF-12 polypeptides of the invention can also be used to produce antibodies which are immunoreactive or bind to epitopes of the GDF-12 polypeptides. Antibody which consists essentially of pooled monoclonal antibodies with different epitopic specificities, as well as distinct monoclonal antibody preparations are provided. Monoclonal antibodies are made from antigen containing fragments of the protein by methods well known in the art

(Kohler, *et al., Nature*, 256:495, 1975; *Current Protocols in Molecular Biology*, Ausubel, *et al.*, ed., 1989).

The term "antibody" as used in this invention includes intact molecules as well as fragments thereof, such as Fab, $F(ab')_2$, and Fv which are capable of binding the epitopic determinant. These antibody fragments retain some ability to selectively bind with its antigen or receptor and are defined as follows:

(1) Fab, the fragment which contains a monovalent antigen-binding fragment of an antibody molecule can be produced by digestion of whole antibody with the enzyme papain to yield an intact light chain and a portion of one heavy chain;

(2) Fab', the fragment of an antibody molecule can be obtained by treating whole antibody with pepsin, followed by reduction, to yield an intact light chain and a portion of the heavy chain; two Fab' fragments are obtained per antibody molecule;

(3) $(Fab')_2$, the fragment of the antibody that can be obtained by treating whole antibody with the enzyme pepsin without subsequent reduction; $F(ab')_2$ is a dimer of two Fab' fragments held together by two disulfide bonds;

(4) Fv, defined as a genetically engineered fragment containing the variable region of the light chain and the variable region of the heavy chain expressed as two chains; and

(5) Single chain antibody ("SCA"), defined as a genetically engineered molecule containing the variable region of the light chain, the variable region of the heavy chain, linked by a suitable polypeptide linker as a genetically fused single chain molecule.

Methods of making these fragments are known in the art. (See for example, Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York (1988), incorporated herein by reference).

As used in this invention, the term "epitope" means any antigenic determinant on an antigen to which the paratope of an antibody binds. Epitopic determinants usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics.

Antibodies which bind to the GDF-12 polypeptide of the invention can be prepared using an intact polypeptide or fragments containing small peptides of interest as the immunizing antigen. The polypeptide or a peptide used to immunize an animal can be derived from translated cDNA or chemical synthesis which can be conjugated to a carrier protein, if desired. Such commonly used carriers which are chemically coupled to the peptide include keyhole limpet hemocyanin (KLH), thyroglobulin, bovine serum albumin (BSA), and tetanus toxoid. The coupled peptide is then used to immunize the animal (e.g., a mouse, a rat, or a rabbit).

If desired, polyclonal or monoclonal antibodies can be further purified, for example, by binding to and elution from a matrix to which the polypeptide or a peptide to which the antibodies were raised is bound. Those of skill in the art will know of various techniques common in the immunology arts for purification and/or concentration of polyclonal antibodies, as well as monoclo-

nal antibodies (See for example, Coligan, *et al.*, Unit 9, *Current Protocols in Immunology*, Wiley Interscience, 1991, incorporated by reference).

It is also possible to use the anti-idiotype technology to produce monoclonal antibodies which mimic an epitope. For example, an anti-idiotypic monoclonal antibody made to a first monoclonal antibody will have a binding domain in the hypervariable region which is the "image" of the epitope bound by the first monoclonal antibody.

The term "cell-proliferative disorder" denotes malignant as well as non-malignant cell populations which often appear to differ from the surrounding tissue both morphologically and genotypically. Malignant cells (i.e. cancer) develop as a result of a multistep process. The GDF-12 polynucleotide that is an antisense molecule is useful in treating malignancies of the various organ systems, particularly, for example, cells in liver tissue. Essentially, any disorder which is etiologically linked to altered expression of GDF-12 could be considered susceptible to treatment with a GDF-12 suppressing reagent. One such disorder is a malignant cell proliferative disorder, for example.

The invention provides a method for detecting a cell proliferative disorder of muscle or adipose tissue which comprises contacting an anti-GDF-12 antibody with a cell suspected of having a GDF-12 associated disorder and detecting binding to the antibody. The antibody reactive with GDF-12 is labeled with a compound which allows detection of binding to GDF-12. For purposes of the invention, an antibody specific for GDF-12 polypeptide may be used to detect the level of GDF-12 in biological fluids and tissues. Any specimen containing a detectable amount of antigen can be used. A preferred sample of this invention is liver tissue. The level of GDF-12 in the suspect cell can be compared with the level in a normal cell to determine whether the subject has a GDF-12-associated cell proliferative disorder. Preferably the subject is human.

The antibodies of the invention can be used in any subject in which it is desirable to administer *in vitro* or *in vivo* immunodiagnosis or immunotherapy. The antibodies of the invention are suited for use, for example, in immunoassays in which they can be utilized in liquid phase or bound to a solid phase carrier. In addition, the antibodies in these immunoassays can be detectably labeled in various ways. Examples of types of immunoassays which can utilize antibodies of the invention are competitive and non-competitive immunoassays in either a direct or indirect format. Examples of such immunoassays are the radioimmunoassay (RIA) and the sandwich (immunometric) assay. Detection of the antigens using the antibodies of the invention can be done utilizing immunoassays which are run in either the forward, reverse, or simultaneous modes, including immunohistochemical assays on physiological samples. Those of skill in the art will know, or can readily discern, other immunoassay formats without undue experimentation.

The antibodies of the invention can be bound to many different carriers and used to detect the presence of an antigen comprising the polypeptide of the invention. Examples of well-known carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses and magnetite. The nature of the carrier can be either soluble or insoluble for purposes of the invention. Those skilled in the art will know of other suitable carriers for binding antibodies, or will be able to ascertain such, using routine experimentation.

There are many different labels and methods of labeling known to those of ordinary skill in the art. Examples of the types of labels which can be used in the present invention include enzymes, radioisotopes, fluorescent compounds, colloidal metals, chemiluminescent compounds, phosphorescent compounds, and bioluminescent compounds. Those of ordinary skill in the art will know of other suitable labels for binding to the antibody, or will be able to ascertain such, using routine experimentation.

Another technique which may also result in greater sensitivity consists of coupling the antibodies to low molecular weight haptens. These haptens can then be specifically detected by means of a second reaction. For example, it is common to use such haptens as biotin, which reacts with avidin, or dinitrophenyl, puridoxal, and fluorescein, which can react with specific anti-hapten antibodies.

In using the monoclonal antibodies of the invention for the *in vivo* detection of antigen, the detectably labeled antibody is given a dose which is diagnostically effective. The term "diagnostically effective" means that the amount of detectably labeled monoclonal antibody is administered in sufficient quantity to enable detection of the site having the antigen comprising a polypeptide of the invention for which the monoclonal antibodies are specific.

The concentration of detectably labeled monoclonal antibody which is administered should be sufficient such that the binding to those cells having the polypeptide is detectable compared to the background. Further, it is desirable that the detectably labeled monoclonal antibody be rapidly cleared from the circulatory system in order to give the best target-to-background signal ratio.

As a rule, the dosage of detectably labeled monoclonal antibody for in vivo diagnosis will vary depending on such factors as age, sex, and extent of disease of the individual. Such dosages may vary, for example, depending on whether multiple injections are given, antigenic burden, and other factors known to those of skill in the art.

For *in vivo* diagnostic imaging, the type of detection instrument available is a major factor in selecting a given radioisotope. The radioisotope chosen must have a type of decay which is detectable for a given type of instrument. Still another important factor in selecting a radioisotope for *in vivo* diagnosis is that deleterious radiation with respect to the host is minimized. Ideally, a radio-

isotope used for *in vivo* imaging will lack a particle emission, but produce a large number of photons in the 140-250 keV range, which may readily be detected by conventional gamma cameras.

For *in vivo* diagnosis radioisotopes may be bound to immunoglobulin either directly or indirectly by using an intermediate functional group. Intermediate functional groups which often are used to bind radioisotopes which exist as metallic ions to immunoglobulins are the bifunctional chelating agents such as diethylenetriaminepentacetic acid (DTPA) and ethylenediaminetetraacetic acid (EDTA) and similar molecules. Typical examples of metallic ions which can be bound to the monoclonal antibodies of the invention are ^{111}In, ^{97}Ru, ^{67}Ga, ^{68}Ga, ^{72}As, ^{89}Zr, and ^{201}Tl.

The monoclonal antibodies of the invention can also be labeled with a paramagnetic isotope for purposes of *in vivo* diagnosis, as in magnetic resonance imaging (MRI) or electron spin resonance (ESR). In general, any conventional method for visualizing diagnostic imaging can be utilized. Usually gamma and positron emitting radioisotopes are used for camera imaging and paramagnetic isotopes for MRI. Elements which are particularly useful in such techniques include ^{157}Gd, ^{55}Mn, ^{162}Dy, ^{52}Cr, and ^{56}Fe.

The monoclonal antibodies of the invention can be used *in vitro* and *in vivo* to monitor the course of amelioration of a GDF-12-associated disease in a subject. Thus, for example, by measuring the increase or decrease in the number of cells expressing antigen comprising a polypeptide of the invention or changes in the concentration of such antigen present in various body fluids, it would be possible to determine whether a particular therapeutic regimen aimed at ameliorating the GDF-12-associated disease is effective. The term "ameliorate" denotes a lessening of the detrimental effect of the GDF-12-associated disease in the subject receiving therapy.

The present invention identifies a nucleotide sequence that can be expressed in an altered manner as compared to expression in a normal cell, therefore it is possible to design appropriate therapeutic or diagnostic techniques directed to this sequence. Thus, where a cell-proliferative disorder is associated with the expression of GDF-12, nucleic acid sequences that interfere with GDF-12 expression at the translational level can be used. This approach utilizes, for example, antisense nucleic acid and ribozymes to block translation of a specific GDF-12 mRNA, either by masking that mRNA with an antisense nucleic acid or by cleaving it with a ribozyme. Such disorders include liver diseases, for example.

Antisense nucleic acids are DNA or RNA molecules that are complementary to at least a portion of a specific mRNA molecule (Weintraub, *Scientific American*, 262:40, 1990). In the cell, the antisense nucleic acids hybridize to the corresponding mRNA, forming a double-stranded molecule. The antisense nucleic acids interfere with the translation of the mRNA, since the cell will not translate a mRNA that is double-stranded. Antisense oligomers of about 15 nucleotides are preferred, since they are easily synthesized and are less likely to cause problems than larger molecules when introduced into the target GDF-12-producing cell. The use of antisense methods to inhibit the *in vitro* translation of genes is well known in the art (Marcus-Sakura, *Anal.Biochem.*, 172:289, 1988).

Ribozymes are RNA molecules possessing the ability to specifically cleave other single-stranded RNA in a manner analogous to DNA restriction endonucleases. Through the modification of nucleotide sequences which encode these RNAs, it is possible to engineer molecules that recognize specific nucleotide sequences in an RNA molecule and cleave it (Cech, *J.Amer.Med. Assn.*, 260:3030, 1988). A major advantage of this approach is that, because they are sequence-specific, only mRNAs with particular sequences are inactivated.

There are two basic types of ribozymes namely, *tetrahymena*-type (Hasselhoff, *Nature*, 334:585, 1988) and "hammerhead"-type. *Tetrahymena*-type ribozymes recognize sequences which are four bases in length, while "hammerhead"-type ribozymes recognize base sequences 11-18 bases in length. The longer the recognition sequence, the greater the likelihood that the sequence will occur exclusively in the target mRNA species. Consequently, hammerhead-type ribozymes are preferable to *tetrahymena*-type ribozymes for inactivating a specific mRNA species and 18-based recognition sequences are preferable to shorter recognition sequences.

The present invention also provides gene therapy for the treatment of cell proliferative or immunologic disorders which are mediated by GDF-12 protein. Such therapy would achieve its therapeutic effect by introduction of the GDF-12 antisense polynucleotide into cells having the proliferative disorder. Delivery of antisense GDF-12 polynucleotide can be achieved using a recombinant expression vector such as a chimeric virus or a colloidal dispersion system. Especially preferred for therapeutic delivery of antisense sequences is the use of targeted liposomes.

Various viral vectors which can be utilized for gene therapy as taught herein include adenovirus, herpes virus, vaccinia, or, preferably, an RNA virus such as a retrovirus. Preferably, the retroviral vector is a derivative of a murine or avian retrovirus. Examples of retroviral vectors in which a single foreign gene can be inserted include, but are not limited to: Moloney murine leukemia virus (MoMuLV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV), and Rous Sarcoma Virus (RSV). A number of additional retroviral vectors can incorporate multiple genes. All of these vectors can transfer or incorporate a gene for a selectable marker so that transduced cells can be identified and generated. By inserting a GDF-12 sequence of interest into the viral vector, along with another gene which encodes the ligand for a receptor on a specific target cell, for example, the vector is now target specific. Retroviral vectors can be made target specific by inserting, for example, a polynucleotide encoding a sugar, a glycolipid, or a protein. Preferred targeting is accomplished by using an antibody to target the retroviral vector. Those of skill in the art will know of, or can readily ascertain without undue experimentation, specific polynucleotide sequences which can be inserted into the retroviral genome to allow target specific delivery of the retroviral vector containing the GDF-12 antisense polynucleotide.

Since recombinant retroviruses are defective, they require assistance in order to produce infectious vector particles. This assistance can be provided, for example, by using helper cell lines that contain plasmids encoding all of the structural genes of the retrovirus under the control of regulatory sequences within the LTR. These plasmids are missing a nucleotide sequence which enables the packaging mechanism to recognize an RNA transcript for encapsidation. Helper cell lines which have deletions of the packaging signal include, but are not limited to Ψ2, PA317 and PA12, for example. These cell lines produce empty virions, since no genome is packaged. If a retroviral vector is introduced into such cells in which the packaging signal is intact, but the structural genes are replaced by other genes of interest, the vector can be packaged and vector virion produced.

Alternatively, NIH 3T3 or other tissue culture cells can be directly transfected with plasmids encoding the retroviral structural genes *gag, pol* and *env*, by conventional calcium phosphate transfection. These cells are then transfected with the vector plasmid containing the genes of interest. The resulting cells release the retroviral vector into the culture medium.

Another targeted delivery system for GDF-12 antisense polynucleotides is a colloidal dispersion system. Colloidal dispersion systems include macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. The preferred colloidal system of this invention is a liposome. Liposomes are artificial membrane vesicles which are useful as delivery vehicles *in vitro* and *in vivo*. It has been shown that large unilamellar vesicles (LUV), which range in size from 0.2-4.0 μm can encapsulate a substantial percentage of an aqueous buffer containing large macromolecules. RNA, DNA and intact virions can be encapsulated within the aqueous interior and be delivered to cells in a biologically active form (Fraley, *et al., Trends Biochem. Sci.*, 6:77, 1981). In addition to mammalian cells, liposomes have been used for delivery of polynucleotides in plant, yeast and bacterial cells. In order for a liposome to be an efficient gene transfer vehicle, the following characteristics should be present: (1) encapsulation of the genes of interest at high efficiency while not compromising their biological activity; (2) preferential and substantial binding to a target cell in comparison to non-target cells; (3) delivery of the aqueous contents of the vesicle to the target cell cytoplasm at high efficiency; and (4) accurate and effective expression of genetic information (Mannino, *et al., Biotechniques*, 6:682, 1988).

The composition of the liposome is usually a combination of phospholipids, particularly high-phase-transition-temperature phospholipids, usually in combination with steroids, especially cholesterol. Other phospholipids or other lipids may also be used. The physical characteristics of liposomes depend on pH, ionic strength, and the presence of divalent cations.

Examples of lipids useful in liposome production include phosphatidyl compounds, such as phosphatidylglycerol, phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, sphingolipids, cerebrosides, and gangliosides. Particularly useful are diacylphosphatidylglycerols, where the lipid moiety contains from 14-18 carbon atoms, particularly from 16-18 carbon atoms, and is saturated. Illustrative phospholipids include egg phosphatidylcholine, dipalmitoylphosphatidylcholine and distearoylphosphatidylcholine.

The targeting of liposomes can be classified based on anatomical and mechanistic factors. Anatomical classification is based on the level of selectivity, for example, organ-specific, cell-specific, and organelle-specific. Mechanistic targeting can be distinguished based upon whether it is passive or active. Passive targeting utilizes the natural tendency of liposomes to distribute to cells of the reticulo-endothelial system (RES) in organs which contain sinusoidal capillaries. Active targeting, on the other hand, involves alteration of the liposome by coupling the liposome to a specific ligand such as a monoclonal antibody, sugar, glycolipid, or protein, or by changing the composition or size of the liposome in order to achieve targeting to organs and cell types other than the naturally occurring sites of localization.

The surface of the targeted delivery system may be modified in a variety of ways. In the case of a liposomal targeted delivery system, lipid groups can be incorporated into the lipid bilayer of the liposome in order to maintain the targeting ligand in stable association with the liposomal bilayer. Various linking groups can be used for joining the lipid chains to the targeting ligand.

Due to the expression of GDF-12 in liver tissue, there are a variety of applications using the polypeptide, polynucleotide, and antibodies of the invention, related to these tissues. Such applications include treatment of cell proliferative disorders involving this tissue. In addition, GDF-12 may be useful in various gene therapy procedures.

The following examples are intended to illustrate but not limit the invention. While they are typical of those that might be used, other procedures known to those skilled in the art may alternatively be used.

EXAMPLE 1
IDENTIFICATION AND ISOLATION OF A NOVEL TGF-β FAMILY MEMBER

To identify novel members of the TGF-β superfamily, degenerate oligonucleotides were designed which corresponded to two conserved regions among the known family members: one region spanning the two tryptophan residues conserved in most family members and the other region spanning the invariant cysteine residues near the C-terminus. These primers were used for polymerase chain reactions on cDNA synthesized from RNA prepared from whole mouse embryos isolated at day 18.5 of gestation. PCR products were subcloned using restriction sites placed at the 5' ends of the primers, and individual bacterial colonies carrying subcloned inserts were screened by a combination of random sequencing and hybridization analysis to eliminate known members of the superfamily.

GDF-12 was identified from a mixture of PCR products obtained with combinations of primer:

SJL218: 5'- CCGGAATTCGGITGG(C/A)G(G/A/T/C)(G/C/A)ATGG (A/G)TI(A/G)TITA(T/C)CC (SEQ ID NO:1)

with each of the following 9 primers:

SJL188: 5'- CCGGAATTC(A/G)CAI(C/G)C(A/G)CAIC(C/T) (G/A/T/C)(T/A)CIACI(G/A)(T/C)CAT-3' (SEQ ID NO:2)

SJL190: 5'- CCGGAATTC(A/G)CAI(C/G)C(A/G)CAIT(C/G) (G/A/T/C)(C/T)GIACI(G/A)(T/C)CAT-3' (SEQ ID NO:3)

SJL191: 5'- CCGGAATTC(A/G)CAI(C/G)C(A/G)CAIT (C/G)(G/A/T/C)(T/A)CIACI(G/A)(T/C)CAT-3' (SEQ ID NO:4)

SJL192: 5'- CCGGAATTC(A/G)CAI(C/G)C(A/G)CAIT(C/G) (G/A/T/C)(C/G/T)TIACI(G/A)(T/C)CAT-3' (SEQ ID NO:5)

SJL193: 5'- CCGGAATTC(A/G)CAI(C/G)C(A/G)CAIG (A/C)(G/A/T/C)(C/T)GIACI(G/A)(T/C)CAT-3' (SEQ ID NO:6)

SJL194: 5'- CCGGAATTC(A/G)CAI(C/G)C(A/G)CAIG (A/C)(G/A/T/C)(T/A)CIACI(G/A)(T/C)CAT-3' (SEQ ID NO:7)

SJL196: 5'- CCGGAATTC(A/G)CAI(C/G)C(A/G)CAI(A/C)G (G/A/T/C)(C/T)GIACI(G/A)(T/C)CAT-3' (SEQ ID NO:8)

SJL197: 5'- CCGGAATTC(A/G)CAI(C/G)C(A/G)CAI (A/C)G(G/A/T/C)(T/A)CIACI(G/A)(T/C)CAT-3' (SEQ ID NO:9)

SJL198: 5'- CCGGAATTC(A/G)CAI(C/G)C(A/G)CAI(A/C)G (G/A/T/C)(C/G/T)TIACI(G/A)(T/C)CAT-3' (SEQ ID NO:10)

PCR using each of these primer combinations was carried out with cDNA prepared from 0.4μg poly A-selected RNA; reactions were carried out at 94°C for 1 minute, 50°C for 2 minutes, and 72°C for 2 minutes for 40 cycles.

PCR products of approximately 280 base pairs were gel purified, digested with *Eco*RI, gel purified again, and subcloned into the Bluescript vector (Stratagene, San Diego, CA). Bacterial colonies carrying individual subclones were picked into 96 well microtiter plates, and multiple replicas were prepared by plating the cells onto nitrocellulose The replicate filters were hybridized to probes representing known members of the family, and DNA was prepared from non-hybridizing colonies for sequence analysis.

Among the colonies analyzed in this manner was one that represented a novel sequence, which was designated GDF-12. This murine sequence was subsequently used to analyze expression patterns and to isolate a human cDNA clone (see below).

EXAMPLE 2

EXPRESSION OF GDF-12

To determine the expression pattern of GDF-12, RNA samples prepared from a variety of adult tissues were screened by Northern analysis. RNA isolation and Northern analysis were carried out as described previously (Lee, S.-J., *Mol. Endocrinol.*, 4:1034, 1990) except that hybridization was carried out in 5X SSPE, 10% dextran sulfate, 50% formamide, 1% SDS, 200 μg/ml salmon DNA, and 0.1% each of bovine serum albumin, ficoll, and polyvinylpyrrolidone. Five micrograms of twice poly A-selected RNA prepared from each tissue were electrophoresed on formaldehyde gels, blotted, and probed with GDF-12. As shown in FIGURE 1, the GDF-12 probe detected a single mRNA species approximately 2.8 and 1.9 kb in length, in adult liver.

EXAMPLE 3

ISOLATION OF cDNA CLONES ENCODING GDF-12

In order to isolate cDNA clones encoding GDF-11, a cDNA library was prepared in the lambda ZAP II vector (Stratagene) using RNA prepared from human adult liver. From 5 μg of twice poly A-selected RNA prepared from human spleen, a cDNA library consisting of 20 million recombinant phage was constructed according to the instructions provided by Stratagene. A portion of this library was screened without amplification using the murine GDF-12 PCR product as a probe. Library screening and characterization of cDNA inserts were carried out as described previously (Lee, *Mol. Endocrinol.*, 4:1034, 1990), except that the final wash was carried out in 2xSSC.

Partial sequence analysis of the first isolated clone showed that it contained the entire coding sequence of GDF-12. A portion of the nucleotide and predicted amino acid sequence of this clone is shown in FIGURE 2 and SEQ ID NOs: 11 and 12. The sequence begins with a putative proteolytic cleavage site which is followed by a C-terminal region of 114 amino acids. The active C-terminal fragment is predicted to have a molecular weight of approximately 12,500.

The entire nucleotide sequence of the longest human GDF-12 cDNA clone isolated is shown in FIGURE 3 and SEQ ID NO:13. The 2419 base pair sequence contains a single long open reading frame beginning with a methionine codon at nucleotides 218-220 and extending for 350 codons. The sequence contains an in-frame stop codon upstream of the putative initiating methionine. The predicted amino acid sequence (SEQ ID NO:14) contains a stretch of hydrophobic amino acids near the N-terminus suggestive of a signal sequence for secretion, one potential N-linked glycosylation site at amino acids 232-236 (box). The C-terminal region following the putative processing site (shaded box) contains all of the hallmarks present in other TGF-β family members (see above).

The C-terminal region following the predicted cleavage site contains all the hallmarks present in other TGF-β family members. GDF-12 contains most of the residues that are highly conserved in other family members, including the seven cysteine residues with their characteristic spacing. Like the TGF-β's, and the inhibin β's, GDF-12 also contains two additional cysteine residues. In the case of TGF-β2, these additional cysteine residues are known to form an intramolecular disulfide bond (Daopin, *et al., Science,* 257:369, 1992; Schlunegger and Grutter, *Nature,* 358:430, 1992). A tabulation of the amino acid sequence homologies between GDF-12 and the other TGF-β family members is shown in FIGURE 4. Numbers represent percent amino acid identities between each pair calculated from the first conserved cysteine to the C-terminus.

Although the invention has been described with reference to the presently preferred embodiment, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: THE JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE

(ii) TITLE OF THE INVENTION: GROWTH DIFFERENTIATION FACTOR-12

(iii) NUMBER OF SEQUENCES: 14

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Fish & Richardson
(B) STREET: 4225 Executive Square, Suite 1400
(C) CITY: La Jolla
(D) STATE: CA
(E) COUNTRY: USA
(F) ZIP: 92037

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ Version 1.5

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: PCT/US95/
(B) FILING DATE: 12-JUL-1995
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Haile, Ph.D., Lisa A
(B) REGISTRATION NUMBER: 38,347
(C) REFERENCE/DOCKET NUMBER: 07265/042WO1 (FD-3830)

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 619-678-5070
(B) TELEFAX: 619-678-5099
(C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(ix) FEATURE:

(A) NAME/KEY: Modified Base
(B) LOCATION: 12...12
(D) OTHER INFORMATION: Inosine

(A) NAME/KEY:Modified Base
(B) LOCATION: 26...26
(D) OTHER INFORMATION: Inosine

(A) NAME/KEY:Modified Base
(B) LOCATION: 29...29
(D) OTHER INFORMATION: Inosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CCGGAATTCG GNTGGMGNVA TGGRTNRTNT AYCC 34

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(ix) FEATURE:

(A) NAME/KEY: Modified Base

(B) LOCATION: 13...13
(D) OTHER INFORMATION: Inosine

(A) NAME/KEY:Modified Base
(B) LOCATION: 19...19
(D) OTHER INFORMATION: Inosine

(A) NAME/KEY:Modified Base
(B) LOCATION: 25...25
(D) OTHER INFORMATION: Inosine; Inosine also at position 28

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CCGGAATTCR CANSCRCANC YNWCNACNRY CAT 33

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(ix) FEATURE:

(A) NAME/KEY: Modified Base
(B) LOCATION: 13...13
(D) OTHER INFORMATION: Inosine

(A) NAME/KEY:Modified Base
(B) LOCATION: 19...19
(D) OTHER INFORMATION: Inosine

(A) NAME/KEY:Modified Base
(B) LOCATION: 25...25
(D) OTHER INFORMATION: Inosine; Inosine also at position 28

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CCGGAATTCR CANSCRCANT SNYGNACNRY CAT 33

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(ix) FEATURE:

(A) NAME/KEY: Modified Base
(B) LOCATION: 13...13
(D) OTHER INFORMATION: Inosine

(A) NAME/KEY:Modified Base
(B) LOCATION: 19...19
(D) OTHER INFORMATION: Inosine

(A) NAME/KEY:Modified Base
(B) LOCATION: 25...25
(D) OTHER INFORMATION: Inosine; Inosine also at position

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CCGGAATTCR CANSCRCANT SNWCNACNRY CAT 33

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(ix) FEATURE:

(A) NAME/KEY: Modified Base
(B) LOCATION: 13...13
(D) OTHER INFORMATION: Inosine

(A) NAME/KEY:Modified Base
(B) LOCATION: 19...19
(D) OTHER INFORMATION: Inosine

(A) NAME/KEY:Modified Base
(B) LOCATION: 25...25
(D) OTHER INFORMATION: Inosine; Inosine also at position 28

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CCGGAATTCR CANSCRCANT SNBTNACNRY CAT 33

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(ix) FEATURE:

(A) NAME/KEY: Modified Base
(B) LOCATION: 13...13
(D) OTHER INFORMATION: Inosine

(A) NAME/KEY:Modified Base
(B) LOCATION: 19...19
(D) OTHER INFORMATION: Inosine

(A) NAME/KEY:Modified Base
(B) LOCATION: 25...25
(D) OTHER INFORMATION: Inosine; Inosine also at position 28

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CCGGAATTCR CANSCRCANG MNYGNACNRY CAT 33

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(ix) FEATURE:

(A) NAME/KEY: Modified Base
(B) LOCATION: 13...13
(D) OTHER INFORMATION: Inosine

(A) NAME/KEY:Modified Base
(B) LOCATION: 19...19
(D) OTHER INFORMATION: Inosine

(A) NAME/KEY:Modified Base
(B) LOCATION: 25...25
(D) OTHER INFORMATION: Inosine; Inosine also at position 28

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CCGGAATTCR CANSCRCANG MNWCNACNRY CAT 33

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(ix) FEATURE:

(A) NAME/KEY: Modified Base
(B) LOCATION: 13...13
(D) OTHER INFORMATION: Inosine

(A) NAME/KEY:Modified Base
(B) LOCATION: 19...19
(D) OTHER INFORMATION: Inosine

(A) NAME/KEY:Modified Base
(B) LOCATION: 25...25
(D) OTHER INFORMATION: Inosine; Inosine also at position 28

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CCGGAATTCR CANSCRCANM GNYGNACNRY CAT 33

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(ix) FEATURE:

(A) NAME/KEY: Modified Base
(B) LOCATION: 13...13
(D) OTHER INFORMATION: Inosine

(A) NAME/KEY:Modified Base
(B) LOCATION: 19...19
(D) OTHER INFORMATION: Inosine

(A) NAME/KEY:Modified Base
(B) LOCATION: 25...25
(D) OTHER INFORMATION: Inosine; Inosine also at position 28

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CCGGAATTCR CAnSCRCANM GNWCNACNMY CAT 33

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(ix) FEATURE:

(A) NAME/KEY: Modified Base
(B) LOCATION: 13...13
(D) OTHER INFORMATION: Inosine

(A) NAME/KEY:Modified Base
(B) LOCATION: 19...19
(D) OTHER INFORMATION: Inosine

(A) NAME/KEY:Modified Base
(B) LOCATION: 25...25
(D) OTHER INFORMATION: Inosine; Inosine also at position 28

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CCGGAATTCR CAnSCRCANM GNWCNACNMY CAT 33

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 360 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(ix) FEATURE:

(A) NAME/KEY: Coding Sequence
(B) LOCATION: 1...357
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
CGG GCC AGG AGG AGG ACC CCC ACC TGT GAG CCT GCG ACC CCC TTA TGT      48
Arg Ala Arg Arg Arg Thr Pro Thr Cys Glu Pro Ala Thr Pro Leu Cys
 1               5                  10                  15

TGC AGG CGA GAC CAT TAC GTA GAC TTC CAG GAA CTG GGA TGG CGG GAC      96
Cys Arg Arg Asp His Tyr Val Asp Phe Gln Glu Leu Gly Trp Arg Asp
            20                  25                  30

TGG ATA CTG CAG CCC GAG GGG TAC CAG CTG AAT TAC TGC AGT GGG CAG     144
Trp Ile Leu Gln Pro Glu Gly Tyr Gln Leu Asn Tyr Cys Ser Gly Gln
        35                  40                  45
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TGC CCT CCC CAC CTG GCT GGC AGC CCA GGC ATT GCT GCC TCT TTC CAT      192
Cys Pro Pro His Leu Ala Gly Ser Pro Gly Ile Ala Ala Ser Phe His
    50                  55                  60

TCT GCC GTC TTC AGC CTC CTC AAA GCC AAC AAT CCT TGG CCT GCC AGT      240
Ser Ala Val Phe Ser Leu Leu Lys Ala Asn Asn Pro Trp Pro Ala Ser
65                  70                  75                  80

ACC TCC TGT TGT GTC CCT ACT GCC CGA AGG CCC CTC TCT CTC CTC TAC      288
Thr Ser Cys Cys Val Pro Thr Ala Arg Arg Pro Leu Ser Leu Leu Tyr
                85                  90                  95

CTG GAT CAT AAT GGC AAT GTG GTC AAG ACG GAT GTG CCA GAT ATG GTG      336
Leu Asp His Asn Gly Asn Val Val Lys Thr Asp Val Pro Asp Met Val
            100                 105                 110

GTG GAG GCC TGT GGC TGC AGC TAG                                     360
Val Glu Ala Cys Gly Cys Ser
        115
```

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acids
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
Arg Ala Arg Arg Arg Thr Pro Thr Cys Glu Pro Ala Thr Pro Leu Cys
 1               5                  10                  15

Cys Arg Arg Asp His Tyr Val Asp Phe Gln Glu Leu Gly Trp Arg Asp
            20                  25                  30

Trp Ile Leu Gln Pro Glu Gly Tyr Gln Leu Asn Tyr Cys Ser Gly Gln
        35                  40                  45

Cys Pro Pro His Leu Ala Gly Ser Pro Gly Ile Ala Ala Ser Phe His
    50                  55                  60
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Ser Ala Val Phe Ser Leu Leu Lys Ala Asn Asn Pro Trp Pro Ala Ser
65 70 75 80

Thr Ser Cys Cys Val Pro Thr Ala Arg Arg Pro Leu Ser Leu Leu Tyr
85 90 95

Leu Asp His Asn Gly Asn Val Val Lys Thr Asp Val Pro Asp Met Val
100 105 110

Val Glu Ala Cys Gly Cys Ser
115

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2419 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(ix) FEATURE:

(A) NAME/KEY: Coding Sequence
(B) LOCATION: 218...1267
(D) OTHER INFORMATION:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GAGCTGTGAG GGTCAAGCAC AGCTATCCAT CAGATGATCT ACTTTCAGCC TTCCTGAGTC 60

CCAGACAATA GAAGACAGGT GGCTGTACCC TTGGCCAAGG GTAGGTGTGG CAGTGGTGTC 120

TGCTGTCACT GTGCCCTCAT TGGCCCCCAG CAATCAGACT CAACAGACGG AGCAACTGCC 180

ATCCGAGGCT CCTGAACCAG GGCCATTCAC CAGGAGC ATG CGG CTC CCT GAT GTC 235
Met Arg Leu Pro Asp Val
1 5

CAG CTC TGG CTG GTG CTG CTG TGG GCA CTG GTG CGA GCA CAG GGG ACA 283
Gln Leu Trp Leu Val Leu Leu Trp Ala Leu Val Arg Ala Gln Gly Thr
10 15 20

```
GGG TCT GTG TGT CCC TCC TGT GGG GGC TCC AAA CTG GCA CCC CAA GCA      331
Gly Ser Val Cys Pro Ser Cys Gly Gly Ser Lys Leu Ala Pro Gln Ala
        25                  30                  35

GAA CGA GCT CTG GTG CTG GAG CTA GCC AAG CAG CAA ATC CTG GAT GGG      379
Glu Arg Ala Leu Val Leu Glu Leu Ala Lys Gln Gln Ile Leu Asp Gly
    40                  45                  50

TTG CAC CTG ACC AGT CGT CCC AGA ATA ACT CAT CCT CCA CCC CAG GCA      427
Leu His Leu Thr Ser Arg Pro Arg Ile Thr His Pro Pro Pro Gln Ala
55                  60                  65                  70

GCG CTG ACC AGA GCC CTC CGG AGA CTA CAG CCA GGG AGT GTG GCT CCA      475
Ala Leu Thr Arg Ala Leu Arg Arg Leu Gln Pro Gly Ser Val Ala Pro
                75                  80                  85

GGG AAT GGG GAG GAG GTC ATC AGC TTT GCT ACT GTC ACA GAC TCC ACT      523
Gly Asn Gly Glu Glu Val Ile Ser Phe Ala Thr Val Thr Asp Ser Thr
            90                  95                  100

TCA GCC TAC AGC TCC CTG CTC ACT TTT CAC CTG TCC ACT CCT CGG TCC      571
Ser Ala Tyr Ser Ser Leu Leu Thr Phe His Leu Ser Thr Pro Arg Ser
        105                 110                 115

CAC CAC CTG TAC CAT GCC CGC CTG TGG CTG CAC GTG CTC CCC ACC CTT      619
His His Leu Tyr His Ala Arg Leu Trp Leu His Val Leu Pro Thr Leu
    120                 125                 130

CCT GGC ACT CTT TGC TTG AGG ATC TTC CGA TGG GGA CCA AGG AGG AGG      667
Pro Gly Thr Leu Cys Leu Arg Ile Phe Arg Trp Gly Pro Arg Arg Arg
135                 140                 145                 150

CGC CAA GGG TCC CGC ACT CTC CTG GCT GAG CAC CAC ATC ACC AAC CTG      715
Arg Gln Gly Ser Arg Thr Leu Leu Ala Glu His His Ile Thr Asn Leu
                155                 160                 165

GGC TGG CAT ACC TTA ACT CTG CCC TCT AGT GGC TTG AGG GGT GAG AAG      763
Gly Trp His Thr Leu Thr Leu Pro Ser Ser Gly Leu Arg Gly Glu Lys
            170                 175                 180

TCT GGT GTC CTG AAA CTG CAA CTA GAC TGC AGA CCC CTA GAA GGC AAC      811
Ser Gly Val Leu Lys Leu Gln Leu Asp Cys Arg Pro Leu Glu Gly Asn
        185                 190                 195

AGC ACA GTT ACT GGA CAA CCG AGG CGG CTC TTG GAC ACA GCA GGA CAC      859
Ser Thr Val Thr Gly Gln Pro Arg Arg Leu Leu Asp Thr Ala Gly His
    200                 205                 210
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CAG CAG CCC TTC CTA GAG CTT AAG ATC CGA GCC AAT GAG CCT GGA GCA      907
Gln Gln Pro Phe Leu Glu Leu Lys Ile Arg Ala Asn Glu Pro Gly Ala
215                 220                 225                 230

GGC CGG GCC AGG AGG AGG ACC CCC ACC TGT GAG CCT GCG ACC CCC TTA      955
Gly Arg Ala Arg Arg Arg Thr Pro Thr Cys Glu Pro Ala Thr Pro Leu
                235                 240                 245

TGT TGC AGG CGA GAC CAT TAC GTA GAC TTC CAG GAA CTG GGA TGG CGG      1003
Cys Cys Arg Arg Asp His Tyr Val Asp Phe Gln Glu Leu Gly Trp Arg
            250                 255                 260

GAC TGG ATA CTG CAG CCC GAG GGG TAC CAG CTG AAT TAC TGC AGT GGG      1051
Asp Trp Ile Leu Gln Pro Glu Gly Tyr Gln Leu Asn Tyr Cys Ser Gly
        265                 270                 275

CAG TGC CCT CCC CAC CTG GCT GGC AGC CCA GGC ATT GCT GCC TCT TTC      1099
Gln Cys Pro Pro His Leu Ala Gly Ser Pro Gly Ile Ala Ala Ser Phe
    280                 285                 290

CAT TCT GCC GTC TTC AGC CTC CTC AAA GCC AAC AAT CCT TGG CCT GCC      1147
His Ser Ala Val Phe Ser Leu Leu Lys Ala Asn Asn Pro Trp Pro Ala
295                 300                 305                 310

AGT ACC TCC TGT TGT GTC CCT ACT GCC CGA AGG CCC CTC TCT CTC CTC      1195
Ser Thr Ser Cys Cys Val Pro Thr Ala Arg Arg Pro Leu Ser Leu Leu
                315                 320                 325

TAC CTG GAT CAT AAT GGC AAT GTG GTC AAG ACG GAT GTG CCA GAT ATG      1243
Tyr Leu Asp His Asn Gly Asn Val Val Lys Thr Asp Val Pro Asp Met
            330                 335                 340

GTG GTG GAG GCC TGT GGC TGC AGC TAGCAAGAGG ACCTGGGGCT TTGGAGTGAA G  1298
Val Val Glu Ala Cys Gly Cys Ser
        345                 350

AGACCAAGAT GAAGTTTCCC AGGCACAGGG CATCTGTGAC TGGAGGCATC AGATTCCTGA   1358

TCCACACCCC AACCCAACAA CCACCTGGCA ATATGACTCA CTTGACCCCT ATGGGACCCA   1418

AATGGGCACT TTCTTGTCTG AGACTCTGGC TTATTCCAGG TTGGCTGATG TGTTGGGAGA   1478

TGGGTAAAGC GTTTCTTCTA AAGGGGTCTA CCCAGAAAGC ATGATTTCCT GCCCTAAGTC   1538

CTGTGAGAAG ATGTCAGGGA CTAGGGAGGG AGGGAGGGAA GGCAGAGAAA AATTACTTAG   1598

CCTCTCCCAA GATGAGAAAG TCCTCAAGTG AGGGGAGGAG GAAGCAGATA GATGGTCCAG   1658
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CAGGCTTGAA GCAGGGTAAG CAGGCTGGCC CAGGGTAAGG GCTGTTGAGG TACCTTAAGG 1718

GAAGGTCAAG AGGGAGATGG GCAAGGCGCT GAGGGAGGAT GCTTAGGGGA CCCCCAGAAA 1778

CAGGAGTCAG GAAAATGAGG CACTAAGCCT AAGAAGTTCC CTGGTTTTTC CCAGGGGACA 1838

GGACCCACTG GGAGACAAGC ATTTATACTT TCTTTCTTCT TTTTTATTTT TTTGAGATCG 1898

AGTCTCGCTC TGTCACCAGG CTGGAGTGCA GTGACACGAT CTTGGCTCAC TGCAACCTCC 1958

GTCTCCTGGG TTCAAGTGAT TCTTCTGCCT CAGCCTCCCG AGCAGCTGGG ATTACAGGCG 2018

CCCACTAATT TTTGTATTCT TAGTAGAAAC GAGGTTTCAA CATGTTGGCC AGGATGGTCT 2078

CAATCTCTTG ACCTCTTGAT CCACCCGACT TGGCCTCCCG AAGTGATGAG ATTATAGGCG 2138

TGAGCCACCG CGCCTGGCTT ATACTTTCTT AATAAAAAGG AGAAAGAAAA TCAACAAATG 2198

TGAGTCATAA AGAAGGGTTA GGGTGATGGT CCAGAGCAAC AGTTCTTCAA GTGTACTCTG 2258

TAGGCTTCTG GGAGGTCCCT TTTCAGGGGT GTCCACAAAG TCAAAGCTAT TTTCATAATA 2318

ATACTAACAT GTTATTTGCC TTTTGAATTC TCATTATCTT AAAATTGTAT TGTGGAGTTT 2378

TCCAGAGGCC GTGTGACATG TGATTACATC ATCTTTCTGA C 2419

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 350 amino acids
(B) TYPE: amino acids
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE: internal
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Met Arg Leu Pro Asp Val Gln Leu Trp Leu Val Leu Leu Trp Ala Leu
1 5 10 15

Val Arg Ala Gln Gly Thr Gly Ser Val Cys Pro Ser Cys Gly Gly Ser
20 25 30

Lys Leu Ala Pro Gln Ala Glu Arg Ala Leu Val Leu Glu Leu Ala Lys
35 40 45

Gln Gln Ile Leu Asp Gly Leu His Leu Thr Ser Arg Pro Arg Ile Thr
50 55 60

His Pro Pro Pro Gln Ala Ala Leu Thr Arg Ala Leu Arg Arg Leu Gln
65 70 75 80

Pro Gly Ser Val Ala Pro Gly Asn Gly Glu Glu Val Ile Ser Phe Ala
85 90 95

Thr Val Thr Asp Ser Thr Ser Ala Tyr Ser Ser Leu Leu Thr Phe His
100 105 110

Leu Ser Thr Pro Arg Ser His His Leu Tyr His Ala Arg Leu Trp Leu
115 120 125

His Val Leu Pro Thr Leu Pro Gly Thr Leu Cys Leu Arg Ile Phe Arg
130 135 140

Trp Gly Pro Arg Arg Arg Arg Gln Gly Ser Arg Thr Leu Leu Ala Glu
145 150 155 160

His His Ile Thr Asn Leu Gly Trp His Thr Leu Thr Leu Pro Ser Ser
165 170 175

Gly Leu Arg Gly Glu Lys Ser Gly Val Leu Lys Leu Gln Leu Asp Cys
180 185 190

Arg Pro Leu Glu Gly Asn Ser Thr Val Thr Gly Gln Pro Arg Arg Leu
195 200 205

Leu Asp Thr Ala Gly His Gln Gln Pro Phe Leu Glu Leu Lys Ile Arg
210 215 220

Ala Asn Glu Pro Gly Ala Gly Arg Ala Arg Arg Arg Thr Pro Thr Cys
225 230 235 240

Glu Pro Ala Thr Pro Leu Cys Cys Arg Arg Asp His Tyr Val Asp Phe
245 250 255

Gln Glu Leu Gly Trp Arg Asp Trp Ile Leu Gln Pro Glu Gly Tyr Gln
260 265 270

Leu Asn Tyr Cys Ser Gly Gln Cys Pro Pro His Leu Ala Gly Ser Pro
275 280 285

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Gly Ile Ala Ala Ser Phe His Ser Ala Val Phe Ser Leu Leu Lys Ala
    290                 295                 300

Asn Asn Pro Trp Pro Ala Ser Thr Ser Cys Cys Val Pro Thr Ala Arg
305                 310                 315                 320

Arg Pro Leu Ser Leu Leu Tyr Leu Asp His Asn Gly Asn Val Val Lys
                325                 330                 335

Thr Asp Val Pro Asp Met Val Val Glu Ala Cys Gly Cys Ser
            340                 345                 350
```

CLAIMS

1. Substantially pure growth differentiation factor-12 (GDF-12) and functional fragments thereof.

2. An isolated polynucleotide sequence encoding the GDF-12 polypeptide of claim 1.

3. The polynucleotide of claim 2, wherein the GDF-12 is selected from the group consisting of:
   a. SEQ ID NO:13, wherein T can also be U;
   b. nucleic acid sequences complementary to SEQ ID NO:13; and
   c. fragments of a. or b. that are at least 15 bases in length and that will selectively hybridize to DNA which encodes the GDF-12 protein of SEQ ID NO:14; and

4. The polynucleotide of claim 2, wherein the polynucleotide is isolated from a mammalian cell.

5. The polynucleotide of claim 4, wherein the mammalian cell is selected from the group consisting of mouse, rat, and human cell.

6. An expression vector including the polynucleotide of claim 2.

7. The vector of claim 6, wherein the vector is a plasmid.

8. The vector of claim 6, wherein the vector is a virus.

9. A host cell stably transformed with the vector of claim 6.

10. The host cell of claim 9, wherein the cell is prokaryotic.

11. The host cell of claim 9, wherein the cell is eukaryotic.

12. Antibodies that bind to the polypeptide of claim 1 or fragments thereof.

13. The antibodies of claim 12, wherein the antibodies are polyclonal.

14. The antibodies of claim 12, wherein the antibodies are monoclonal.

15. A method of detecting a cell proliferative disorder comprising contacting the antibody of claim 12 with a specimen of a subject suspected of having a GDF-12 associated disorder and detecting binding of the antibody.

16. The method of claim 15, wherein the cell is a liver cell.

17. The method of claim 15, wherein the detecting is *in vivo*.

18. The method of claim 17, wherein the antibody is detectably labeled.

19. The method of claim 18, wherein the detectable label is selected from the group consisting of a radioisotope, a fluorescent compound, a bioluminescent compound and a chemiluminescent compound.

20. The method of claim 15, wherein the detection is *in vitro*.

21. The method of claim 20, wherein the antibody is detectably labeled.

22. The method of claim 21, wherein the label is selected from the group consisting of a radioisotope, a fluorescent compound, a bioluminescent compound, a chemoluminescent compound and an enzyme.

23. A method of treating a cell proliferative disorder associated with expression of GDF-12, comprising contacting the cells with a reagent which suppresses the GDF-12 activity.

24. The method of claim 23, wherein the reagent is an anti-GDF-12 antibody.

25. The method of claim 23, wherein the reagent is a GDF-12 antisense sequence.

26. The method of claim 23, wherein the cell is a liver cell.

27. The method of claim 23, wherein the reagent which suppresses GDF-12 activity is introduced to a cell using a vector.

28. The method of claim 27, wherein the vector is a colloidal dispersion system.

29. The method of claim 28, wherein the colloidal dispersion system is a liposome.

30. The method of claim 29, wherein the liposome is essentially target specific.

31. The method of claim 30, wherein the liposome is anatomically targeted.

32. The method of claim 31, wherein the liposome is mechanistically targeted.

33. The method of claim 32, wherein the mechanistic targeting is passive.

34. The method of claim 32, wherein the mechanistic targeting is active.

35. The method of claim 34, wherein the liposome is actively targeted by coupling with a moiety selected from the group consisting of a sugar, a glycolipid, and a protein.

36. The method of claim 35, wherein the protein moiety is an antibody.

37. The method of claim 36, wherein the vector is a virus.

38. The method of claim 37, wherein the virus is an RNA virus.

39. The method of claim 38, wherein the RNA virus is a retrovirus.

40. The method of claim 39, wherein the retrovirus is essentially target specific.

41. The method of claim 40, wherein a moiety for target specificity is encoded by a polynucleotide inserted into the retroviral genome.

42. The method of claim 40, wherein a moiety for target specificity is selected from the group consisting of a sugar, a glycolipid, and a protein.

43. The method of claim 42, wherein the protein is an antibody.

ovary
liver
muscle
testis
spleen
intestine
pancreas
seminal vesicle
kidney
brain
thymus
lung
heart

FIG. 1

```
1   CGGGCCAGGAGGAGGACCCCCACCTGTGAGCCTGCGACCCCCTTATGTTGCAGGCGAGAC   60
    R  A  R  R  R  T  P  T  C  E  P  A  T  P  L  C  C  R  R  D
61  CATTACGTAGACTTCCAGGAACTGGGATGGCGGGACTGGATACTGCAGCCCGAGGGGTAC  120
    H  Y  V  D  F  Q  E  L  G  W  R  D  W  I  L  Q  P  E  G  Y
121 CAGCTGAATTACTGCAGTGGGCAGTGCCCTCCCCACCTGGCTGGCAGCCCAGGCATTGCT  180
    Q  L  N  Y  C  S  G  Q  C  P  P  H  L  A  G  S  P  G  I  A
181 GCCTCTTTCCATTCTGCCGTCTTCAGCCTCCTCAAAGCCAACAATCCTTGGCCTGCCAGT  240
    A  S  F  H  S  A  V  F  S  L  L  K  A  N  N  P  W  P  A  S
241 ACCTCCTGTTGTGTCCCTACTGCCCGAAGGCCCCTCTCTCTCCTCTACCTGGATCATAAT  300
    T  S  C  C  V  P  T  A  R  R  P  L  S  L  L  Y  L  D  H  N
301 GGCAATGTGGTCAAGACGGATGTGCCAGATATGGTGGTGGAGGCCTGTGGCTGCAGCTAG  360
    G  N  V  V  K  T  D  V  P  D  M  V  V  E  A  C  G  C  S  *
```

FIG. 2

Family member	% identity with GDF-12
GDF-1	43
GDF-3	36
GDF-5	36
GDF-6	39
GDF-7	42
GDF-9	30
BMP-3	37
BMP-2	43
BMP-4	42
Vgr-1	41
OP-1	40
BMP-5	38
OP-2	39
MIS	30
Inhibin-α	27
Inhibin-βA	47
Inhibin-βB	50
Nodal	38
GDNF	21
TGF-β1	36
TGF-β2	36
TGF-β3	41

FIG. 4

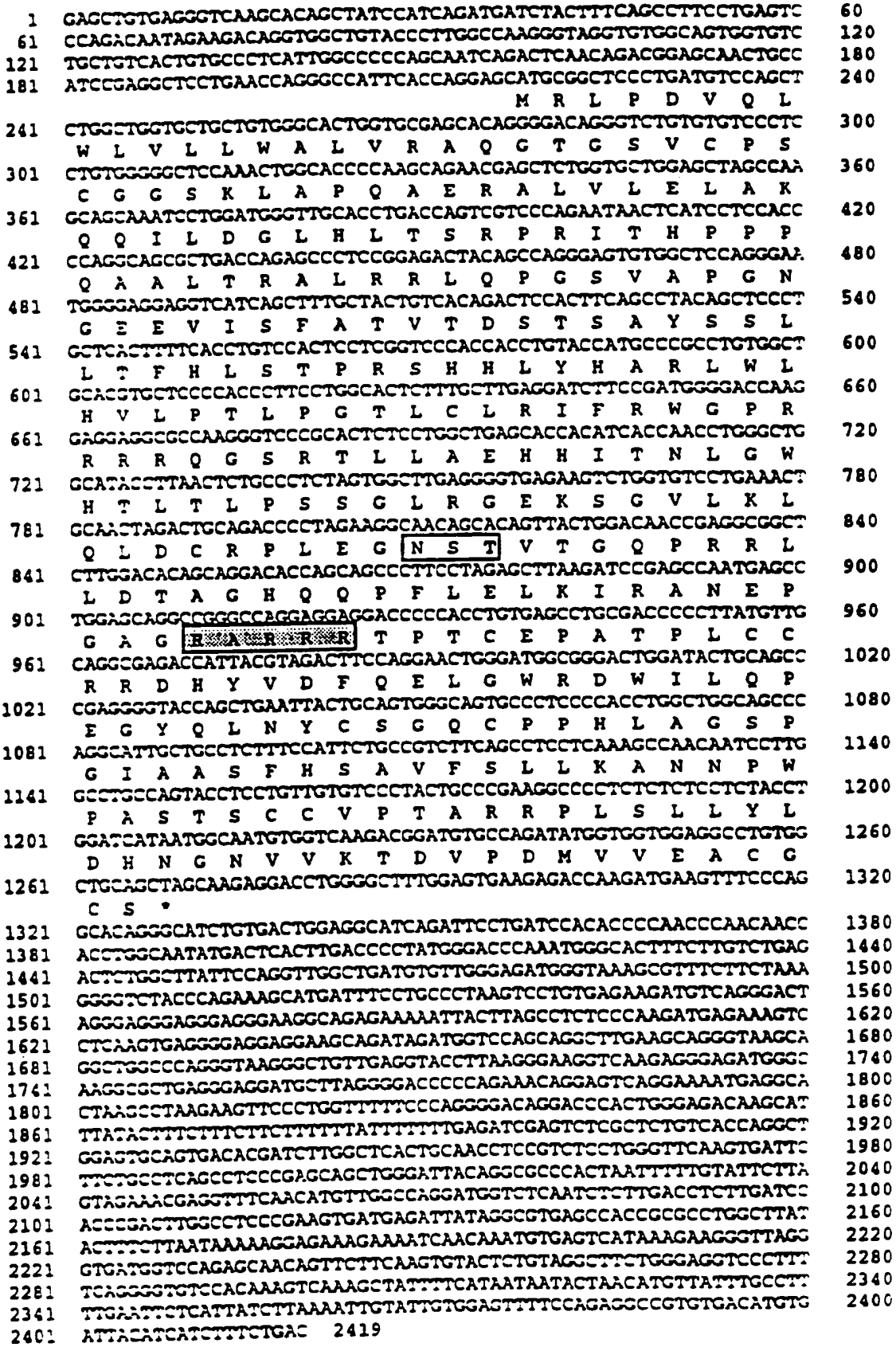

FIG. 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/08745

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07H 21/04; C12N 1/20, 15/09, 15/18, 15/63, 15/64, 15/66
US CL :530/350, 399; 536/23.1; 435/69.1, 252.3, 320.1, 172.3
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/350, 399; 536/23.1; 435/69.1, 252.3, 320.1, 172.3

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CAS ONLINE, MEDLINE, BIOSIS, EMBASE, SCISEARCH
search terms: growth differentiation factor, production or isolation, mammal, sequence, polynucleotide, cloning

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCE, U.S.A., Volume 90, issued July 1993, Sampath et al, "Drosophila Transforming Growth Factor Beta Superfamily Proteins Induce Endochondral Bone Formation in Mammals", pages 6004-6008, see page 6004, column 1, see abstract.	1-11
A	BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, Volume 204, Number 2, issued 28 October 1994, Hotten et al, "Cloning and Expression of Recombinant Human Growth/Differentiation Factor 5", pages 646-652, see page 646, see abstract.	1-11
A	WO, A, 93/16099 (NEIDHARDT ET AL) 19 August 1993, page 3, lines 10-13.	1-11

☐ Further documents are listed in the continuation of Box C ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
03 OCTOBER 1995	26 OCT 1995
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer D. Fries for PREMA MERTZ Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/08745

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-11

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/08745

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

I. Claims 1-11, drawn to a growth differentiation factor-12 (GDF-12), a polynucleotide sequence encoding the GDF-12, an expression vector and a host cell.
II. Claims 12-22, drawn to antibodies, and a method of detecting a cell proliferative disorder by contacting the antibody with a specimen.
III. Claims 23-24, and 26-43, drawn to a method of treating a cell proliferative disorder by contacting cells with anti-GDF-12 antibody.
IV. Claims 23, and 25-43, drawn to a method of treating a cell proliferative disorder by contacting cells with GDF-12 antisense sequence.
The inventions listed as Groups I-IV do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Groups I-II are drawn to separate, distinct inventions and are distinguished from each other because the special technical features which define them by chemical and physical characteristics as well as biological functions are different and these special technical features are not shared by each invention. Since these special technical features are not shared by each product and since the common features do not establish an advance over the prior art, the inventions of Groups I-II do not form a single inventive concept within the meaning of Rule 13.2.
Groups III-IV are drawn to methods having different methodsteps,andreagents which do not share the same or a corresponding special technical feature which define the contribution of each invention. Since these special technical features is not shared by each process and since the common features do not establish an advance over the prior art, the inventions of Groups III-IV do not form a single inventive concept within the meaning of Rule 13.2.
The invention of Group I is separate and distinct from the inventions of Groups III and IV because the invention of Group I is not used or produced by the inventions of Groups III and IV.
The invention of Group II is separate and distinct from the invention of Group IV because the invention of Group II is not used or produced by the invention of Group IV.
The invention of Group II is separate and distinct from the invention of Group III because the invention of Group II may be used in other methods other than the method of treating a cell proliferative disorder. The antibody of Group II can be used in immunodiagnostics.